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类叶升麻具有毛茛科中最对称和最原始的核型吗?*

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DOES *ACTAEA ASIATICA* HAVE THE MOST SYMMETRIC AND PRIMITIVE KARYOTYPE IN THE RANUNCULACEAE?

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Abstract *Actaea asiatica* was previously reported to have the most symmetric and primitive karyotype, consisting of 10 m- and 6 sm-chromosomes, which is quite different from those of the remaining species in the genus *Actaea*, consisting of 10 m-, 4 sm- and two T-chromosomes. In this paper, the chromosomes of this species were re-examined. The results show that *Actaea asiatica* has the same karyotype as the other species in the genus. Compared with the species in other genera in the tribe Cimicifugeae, i. e. *Beesia*, *Anemonopsis*, *Souliea* and *Cimicifuga*, *Actaea asiatica*, together with the remaining species of the genus, has the most asymmetric and thus probably the most advanced karyotype in this tribe because of the presence of two T-chromosomes in their chromosome complements. The two T-chromosomes may serve as one of the most important cytological markers, by which the species in *Actaea* are clearly distinguishable cytologically from those in *Beesia*, *Anemonopsis*, *Souliea* and *Cimicifuga*.

Key words *Actaea asiatica*; Karyotype; Ranunculaceae

Actaea L. is a small genus of eight species in the Ranunculaceae (Tamura, 1995). All the species have been cytologically studied and found to have the same chromosome number of 2n = 16 (Wang *et al*., 1994; Kurosawa, 1979; Kawano *et al*., 1966; Kurita, 1959, 1957). Their karyotypes are also essentially uniform, consisting of 10 large m-, 4 moderately large sm- and 2 smaller T-chromosomes. The smallest pair of T-chromosomes, which have no short arms, are very remarkable and may serve as one of the most important cytological markers of the genus, by which the species in this genus can be clearly distinguished cytologically from the other species in the tribe Cimicifugeae, which, besides *Actaea*, includes *Beesia*, *Anemonopsis*, *Souliea*, *Cimicifuga* (W. T. Wang, 1979). In the latter 4 genera, the smallest pair of chromosomes have very short but always clearly observable short arms (Yang, 1995; Hasegawa & Peng, 1991; Hasegawa, 1970a, b, c, 1969; Kurita, 1957). However, the karyotype of *A. asiatica* was repeatedly reported to comprise 10 m- and 6 sm-chromosomes, with the smallest pair having submedian centromeres (Wang *et al*., 1994; Kurita, 1957). This result has unfortunately led Wang *et al*. (1994) to consider that *A. asiatica* is cytologically quite distinct from its close relative, *A. erythrocarpa*, which has the karyotype

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of 10 m-, 4 sm- and 2 T-chromosomes, and more seriously, further led Kurita(1958) and Tamura (1995) to believe that *A. asiatica* might have the most symmetric and thus the most primitive karyotype in the Ranunculaceae, although simultaneously Tamura felt a little confused about this result and pointed out that it is far from understandable, because *A. asiatica* has as the other species in the genus the same berry-like follicles, a very specialized gross-morphological character of the genus which indicates its advanced position in the tribe Cimicifugeae. As is well-known, the other 4 genera, i. e. *Beesia*, *Anemonopsis*, *Souliea* and *Cimicifuga*, all have typical follicles, which are usually regarded as a primitive character. Having studied the chromosomes of the Ranunculaceae for many years and found that the chromosome morphology in a same genus in this family is often basically quite uniform, I guess that Kurita's and Wang *et al.*'s reports of the karyotype of *A. asiatica* mentioned above are most probably wrong. As seen from their papers, the chromosomes in their preparations are not so well-spread and the chromosome morphology is not good enough to allow making exact karyotypic analysis. In order to confirm my conjecture, the chromosomes of this species were here re-examined.

1 Materials and Methods

Two populations, one from Huadianba, Cangshan Mountain, Dali City, Yunnan Province, the other from Yezhi, Yunling Mountain, Weixi County, also Yunnan Province, were collected from the field and then transplanted to the greenhouse of the Botanic Garden, Kunming Institute of Botany, the Chinese Academy of Sciences. The voucher specimens, Yang Qin-er 9430 and 9477, were deposited in the Herbarium, Institute of Botany, the Chinese Academy of Sciences (PE).

For the observation of the chromosomes, actively growing roots were harvested and then pretreated with 0.1% colchcine for 2.5 hours at room temperature. They were then fixed in Carnoy's fluid (absolute ethanol: glacial acetic acid = 3:1) at 4 ℃ for 30 minutes. After being macerated in the mixture of 1 mol/L HCl and 45% acetic acid at 60 ℃ for three minutes, they were stained in 1% aceto-orcein and squashed.

Karyomorphological classification of resting nuclei and mitotic prophase chromosomes followed Tanaka (1977, 1971). The symbols for the description of karyotypes followed Levan *et al.* (1964).

2 Results

In resting nuclei (Fig. 1: A), numerous chromocenters were observed. The other regions were also stained well but unevenly. Thus, the resting nuclei were categorized as the complex chromocenter type.

In prophase chromosomes (Fig. 1: B), hetero- and euchromatic segments were distinguishable but their boundaries were not distinct and the heterochromatic segments were distributed in the distal and interstitial as well as proximal regions. Thus, the prophase chromosomes were categorized as the interstitial type.

The metaphase chromosomes of both populations were counted to be 2n = 16 (Fig. 1: C, D, E, F). Their karyotypes were very similar and were formulated as 2n = 10m(3sat) + 4sm + 2T(Table 1). Five pairs (the first, the second, the third, the fourth and the fifth) of chromosomes were quite large and had median centromeres, and two pairs (the sixth and the seventh) were moderately large and had submedian centromeres, and the last and smallest pair had no short arms and thus belonged to T-chromosomes. The first, the fifth and the sixth chromosomes had relatively large satellites on the short arms.

Table 1 Parameters of chromosomes in two populations of *Actaea asiatica*

Chromosome No.	Population from Dali (Yang Qin-er 9430)			Population from Weixi (Yang Qin-er 9477)		
	Relative length	Arm ratio	Type	Relative length	Arm ratio	Type
1	4.59+3.65=8.24	1.26	m*	4.18+3.67=7.85	1.14	m*
2	4.42+3.53=7.95	1.25	m	4.18+3.67=7.85	1.14	m
3	4.06+3.77=7.83	1.08	m	4.05+3.54=7.59	1.14	m
4	4.14+3.61=7.75	1.15	m	4.18+3.29=7.47	1.27	m
5	3.98+3.73=7.71	1.08	m*	3.92+3.54=7.46	1.11	m*
6	3.65+3.25=6.90	1.12	m*	3.92+3.54=7.46	1.11	m*
7	3.65+3.04=6.69	1.20	m	3.80+3.04=6.84	1.25	m
8	3.65+2.84=6.49	1.29	m	3.80+3.04=6.84	1.25	m
9	3.81+2.64=6.45	1.44	m	3.54+2.78=6.32	1.27	m
10	3.45+2.80=6.25	1.23	m	3.54+2.78=6.32	1.27	m
11	3.49+2.03=5.52	1.72	sm	3.67+2.03=5.70	1.81	sm
12	3.41+1.78=5.19	1.92	sm	3.67+2.03=5.70	1.81	sm
13	3.04+1.42=4.46	2.14	sm	3.04+1.52=4.56	2.00	sm
14	3.04+1.42=4.46	2.14	sm	2.66+1.52=4.18	1.75	sm
15	4.02+0.00=4.02	∞	T	3.92+0.00=3.92	∞	T
16	3.94+0.00=3.94	∞	T	3.92+0.00=3.92	∞	T

* indicates sat-chromosomes. The length of satellites is included in short arms.

3 Discussion

The present results clearly show that the karyotype of *Actaea asiatica* has basically the same karyotype as the other species in this genus, and the smallest pair of chromosomes have no short arms. Thus we can safely say that both the result of karyotypic analysis of *Actaea asiatica* reported by Kurita(1957) and that by Wang *et al*. (1994) are wrong. They all mistakenly recognized the smallest pair as sm-chromosomes due to the poor quality of their chromosome preparations on the one hand, and perhaps more importantly, due to their lacking of an overall understanding of the chromosome morphology and gross-morpholoical characters of the Ranunculaceae. If they had known that in the Ranunculaceae the chromosome morphology in a same genus is often uniform, which actually is the reason why the chromosomal evidence is of utmost importance to determine the systematic positions of the genera in the family as repeatedly stressed by Tamura(1995, 1984), and that the genus *Actaea* is phylogenetically very specialized in the family as indicated by its having berry-like follicles, such mistakes should have been avoidable. Indeed, in cytotaxonomic research of plants, the importance of high-quality chromosome preparations and a comprehensive view of the chromosomal and gross-morphological characteristics of the group under question cannot be too highly emphasized.

As mentioned above, the tribe Cimicifugeae includes five genera, i. e. *Beesia*, *Anemonopsis*, *Souliea*, *Cimicifuga* and *Actaea* and all of them have been cytologically studied. In general, they have very similar karyomorphological characteristics compared with the other groups in the Ranunculaceae. Their chromosomes are quite large, with the five largest pairs having median or submedian centromeres, the two moderately large pairs having submedian or subterminal centromeres depending on the species, and the smallest pair having

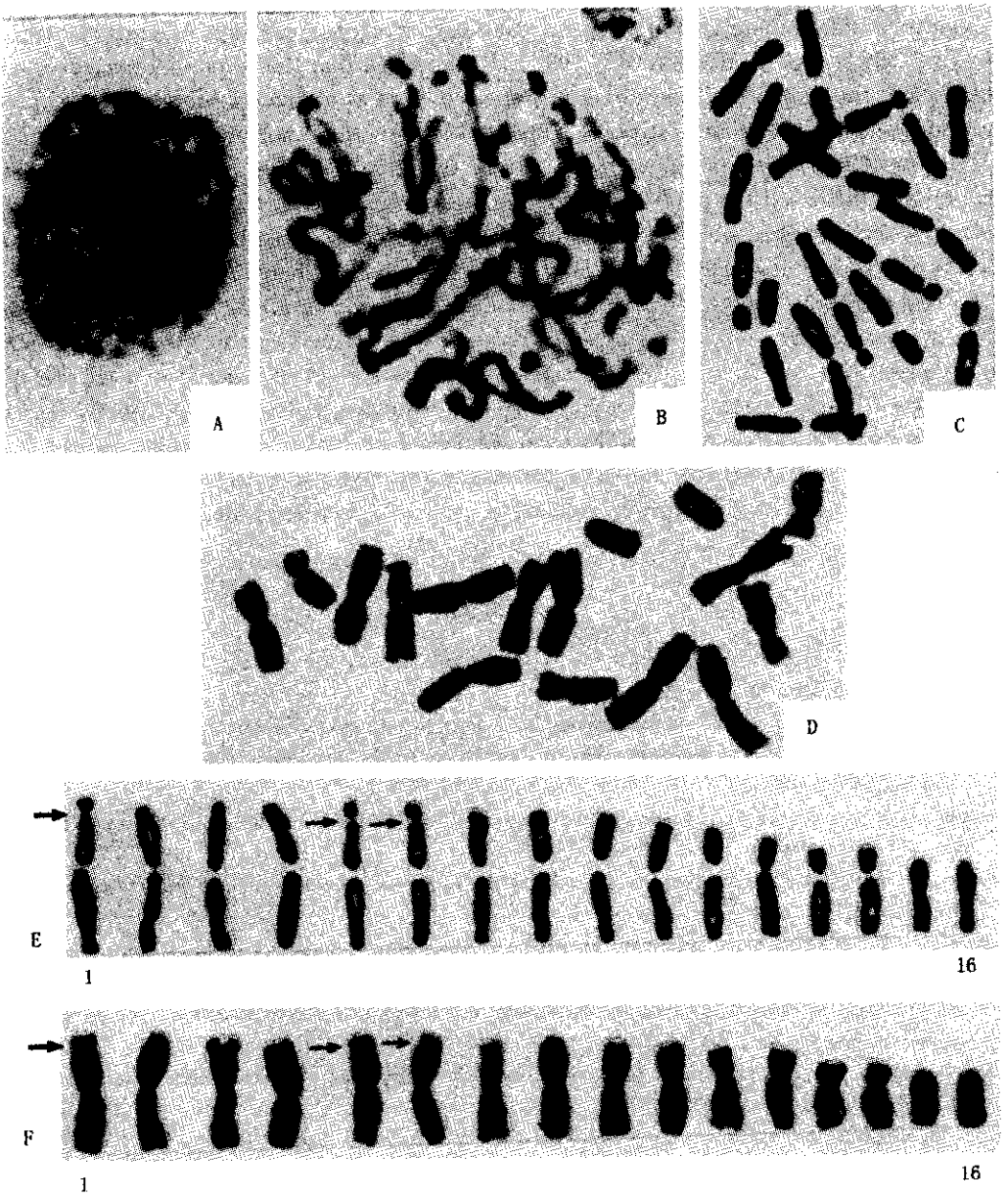

Fig. 1　Photomicrographs of resting nuclei, prophase chromosomes and metaphase chromosomes in two populations of *Actaea asiatica* Hara

A, B, C and E——population from Dali(Yang Qin-er 9430); D and F—— population from Weixi(Yang Qin-er 9477). A. resting nuclei; B. prophase chromosomes; C～F. metaphase chromosomes. (all ×1940; the arrows indicate the secondary constrictions.)

short but always clearly observable arms and terminal centromeres (in *Beesia*, *Anemonopsis*, *Souliea* and *Cimicifuga*), or simply having no short arms(in *Actaea*). In the Ranunculaceae, such combinations of karyotypic characters are only found in this tribe. It is also noteworthy that the secondary constrictions seem to much more frequently appear in this tribe, particularly in the genus *Actaea*, than in the other groups of the Ranunculaceae.

Hence, karyomorphologically the tribe Cimicifugeae is fairly isolated in the family. Based on the evidence from serology, phytochemistry and palynology respectively, Hammond (1952), Hsiao (1980) as well as Wang *et al*. (1993) all contended that this tribe should be better removed from the subfamily Helleboroideae and established as a separate subfamily, i.e. Cimicifugoideae. This viewpoint is strongly supported by karyomorphological data.

In the Ranunculaceae, *Cimicifuga* may be the closest relative of *Actaea*. The former has typical follicles but the latter has specialized berry-like follicles. Cytologically *Cimicifuga* has two st-chromosomes but *Actaea* has two T-chromosomes. It seems that both gross-morphologically and cytologically *Actaea* may be more specialized than *Cimicifuga*.

From the above analyses, it can be concluded that *Actaea asiatica* has basically the same karyotype as the other species in the genus *Actaea*, and its smallest pair (the eighth pair) of chromosomes have no short arms. Compared with the species in other genera of the tribe Cimicifugeae, *Actaea asiatica*, together with the remaining species in the genera, has the most asymmetric and thus probably the most specialized karyotype in the tribe, instead of the most symmetric and primitive one. The specialized karyotypic character of this genus might be correlated to some degree with its specialized gross-morphological character, the berry-like follicles.

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摘要　根据以前的报道，类叶升麻 *Actaea asiatica* Hara 具有 10 条大型的中部着丝点染色体和 6 条较大的近中部着丝点染色体，其核型在毛茛科中显得最为对称和原始，而类叶升麻属的其他种类具有 10 条大型的中部着丝点染色体、4 条较大的近中部着丝点染色体和两条没有短臂的染色体。在毛茛科中，同一属的染色体形态通常十分相似，因此上述类叶升麻的核型分析结果十分可疑。本文重新检查了该种的染色体。结果表明其核型与该属其他种类的核型没有明显区别。与升麻属其他 4 属，即 *Beesia*, *Anemonopsis*, *Souliea*, *Cimicifuga* 相比，类叶升麻及该属其他种类都具有两条没有短臂的 T 染色体，因此类叶升麻属 *Actaea* L. 的核型不对称性程度在升麻族中显得最高，其核型在该族中也可能最为进化。这两条 T 染色体可以作为类叶升麻属的细胞学标志，据此可以将该属与升麻族其他 4 属区别开来。

关键词　类叶升麻；核型；毛茛科